Anti-alpha Actinin Antibody [A1G2]

EM1901-52

Product Type:	Mouse monoclonal IgG1, primary antibodies
Species reactivity:	Human, Rat
Applications:	WB, IF-Cell, IHC-P, FC
Molecular Wt:	Predicted band size: 103 kDa
Clone number:	A1G2

Description: Alpha actinins belong to the spectrin gene superfamily which represents a diverse group of cytoskeletal proteins, including the alpha and beta spectrins and dystrophins. Alpha actinin is an actin-binding protein with multiple roles in different cell types. In nonmuscle cells, the cytoskeletal isoform is found along microfilament bundles and adherens-type junctions, where it is involved in binding actin to the membrane. In contrast, skeletal, cardiac, and smooth muscle isoforms are localized to the Z-disc and analogous dense bodies, where they help anchor the myofibrillar actin filaments. This gene encodes a nonmuscle, cytoskeletal, alpha actinin isoform and maps to the same site as the structurally similar erythroid beta spectrin gene. Three transcript variants encoding different isoforms have been found for this gene.

Immunogen: Recombinant protein within Human ACTN1 aa 388-619 / 892.

Positive control: HeLa cell lysate, A431 cell lysate, A549 cell lysate, MCF7 cell lysate, PC-12 cell lysate, C6 cell lysate, A431, SiHa, JAR, human lung tissue, human liver carcinoma tissue, human skin tissue, human breast tissue, human breast carcinoma tissue, human kidney tissue.

Subcellular location: Cell membrane, cytoskeleton, Z line, cell junction, ruffle.

Database links: SwissProt: P12814 Human | Q9Z1P2 Rat

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:50-1:100
IHC-P	1:50-1:500
FC	1:50-1:100

Storage Buffer: 1*PBS (pH7.4), 0.2% BSA, 50% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

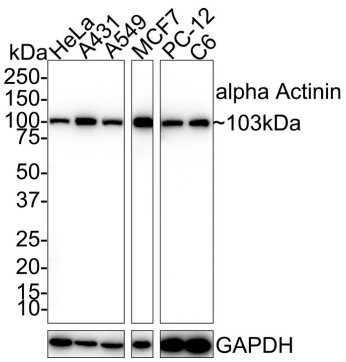

Fig1: Western blot analysis of alpha Actinin on different lysates with Mouse anti-alpha Actinin antibody (EM1901-52) at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: A431 cell lysate
Lane 3: A549 cell lysate
Lane 4: MCF7 cell lysate
Lane 5: PC-12 cell lysate
Lane 6: C6 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 103 kDa
Observed band size: 103 kDa

Exposure time: 1 minute;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (EM1901-52) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

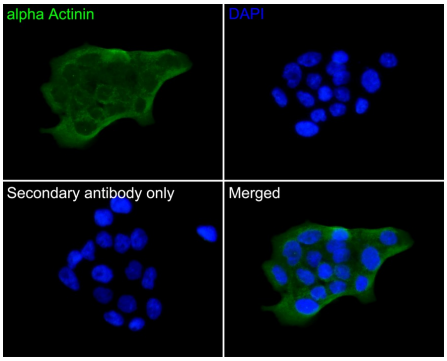

Fig2: Immunocytochemistry analysis of A431 cells labeling alpha Actinin with Mouse anti-alpha Actinin antibody (EM1901-52) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 30 minutes, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes, and then blocked with 2% BSA for 30 minutes at room temperature. Cells were then incubated with Mouse anti-alpha Actinin antibody (EM1901-52) at 1/50 dilution in 2% BSA overnight at 4 ℃. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

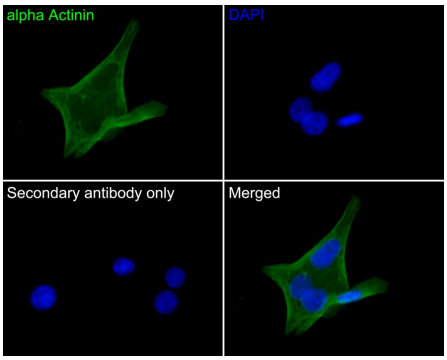

Fig3: Immunocytochemistry analysis of SiHa cells labeling alpha Actinin with Mouse anti-alpha Actinin antibody (EM1901-52) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 30 minutes, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes, and then blocked with 2% BSA for 30 minutes at room temperature. Cells were then incubated with Mouse anti-alpha Actinin antibody (EM1901-52) at 1/50 dilution in 2% BSA overnight at 4 ℃. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

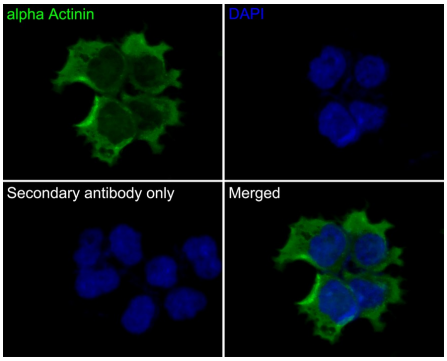

Fig4: Immunocytochemistry analysis of JAR cells labeling alpha Actinin with Mouse anti-alpha Actinin antibody (EM1901-52) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 30 minutes, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes, and then blocked with 2% BSA for 30 minutes at room temperature. Cells were then incubated with Mouse anti-alpha Actinin antibody (EM1901-52) at 1/50 dilution in 2% BSA overnight at 4 ℃. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

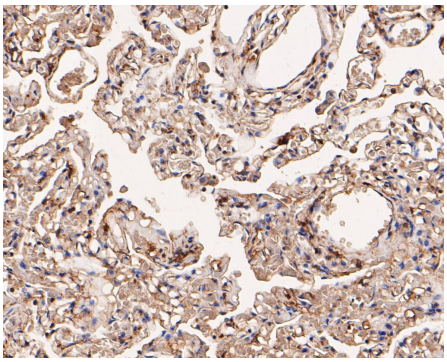

Fig5: Immunohistochemical analysis of paraffin-embedded human lung tissue using anti-alpha Actinin antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (EM1901-52, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

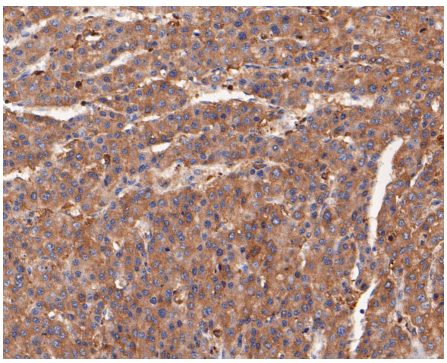

Fig6: Immunohistochemical analysis of paraffin-embedded human liver carcinoma tissue using anti-alpha Actinin antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (EM1901-52, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

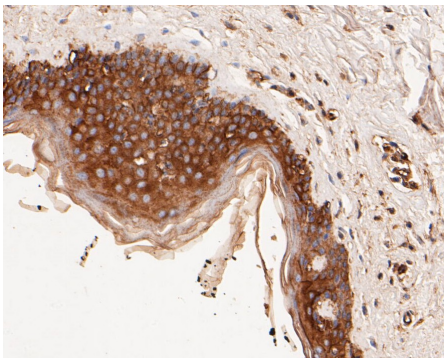

Fig7: Immunohistochemical analysis of paraffin-embedded human skin tissue using anti-alpha Actinin antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (EM1901-52, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

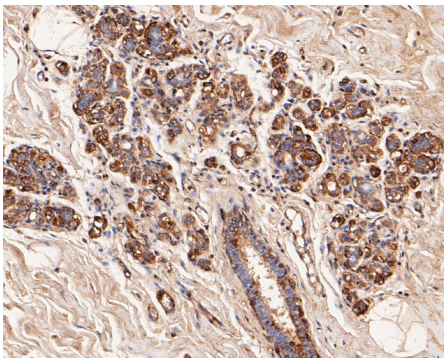

Fig8: Immunohistochemical analysis of paraffin-embedded human breast tissue using anti-alpha Actinin antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (EM1901-52, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

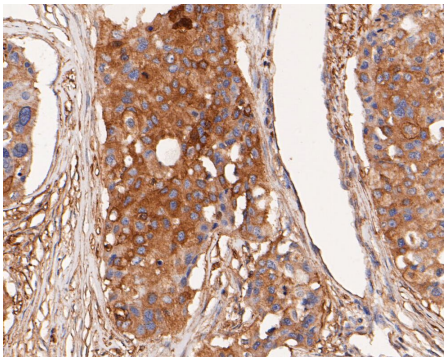

Fig9: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using anti-alpha Actinin antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (EM1901-52, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

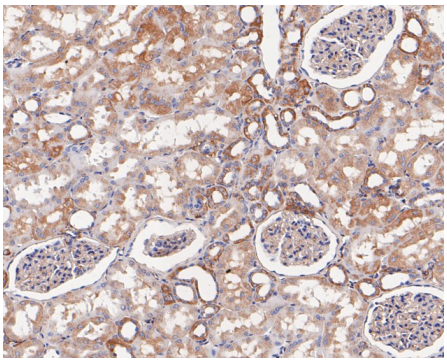

Fig10: Immunohistochemical analysis of paraffin-embedded human kidney tissue using anti-alpha Actinin antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (EM1901-52, 1/200) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

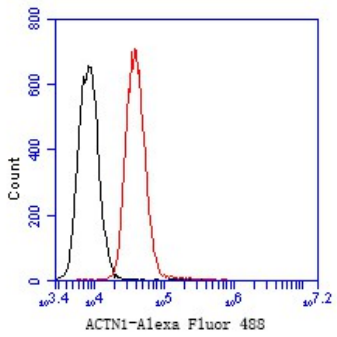

Fig11: Flow cytometric analysis of alpha Actinin was done on A431 cells. The cells were fixed, permeabilized and stained with the primary antibody (EM1901-52, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Mouse IgG Secondary antibody at 1/1000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Kunishima S. et. al. ACTN1 mutations cause congenital macrothrombocytopenia. Am. J. Hum. Genet. 92:431-438(2013).
2. Gueguen P. et. al. A missense mutation in the alpha-actinin 1 gene (ACTN1) is the cause of autosomal dominant macrothrombocytopenia in a large French family. PLoS ONE 8:E74728-E74728(2013).